Claims

What is claimed is:

1. A method of detecting DNA variation by monitoring the formation or dissociation of a complex consisting of:

   (a) a single strand of a DNA sequence containing the locus of a variation,

   (b) an oligonucleotide or DNA analogue probe specific for one allele of the variation and capable of hybridizing to the single strand (a) to form a duplex,

   (c) a marker specific for the duplex structure of (a) plus (b) which forms a complex with the said duplex and reacts uniquely when interacting within the duplex,

   which method comprises:

   (1) continually measuring an output signal indicative of interaction of the marker with duplex formed from the strand (a) and probe (b), and

   (2) recording the conditions at which a change in reaction output signal occurs which is attributable to formation or dissociation of the complex and is thereby correlated with the strength with which the probe (b) has hybridized to the single strand (a).

2. A method according to claim 1 including

   (1) forming a series of two or more complexes of the kind defined, each with a probe specific for a different allele of the variation, and

   (2) observing their respective denaturing or annealing conditions (e.g. denaturing or annealing temperatures) so as to distinguish alleles of the variation plus the homozygous or heterozygous state if appropriate.

3. A method according to claim 1, in which the marker is one which fluoresces when intercalated in double stranded DNA.

4. A method according to claim 3, in which the denaturing or annealing point is determined by reference to the first derivative of the fluorescence measurement curve.

5. A method according to claim 3, in which denaturing or annealing point is determined by reference to the second derivative of the fluorescence measurement curve.

6. A method according to claim 1, in which the single strand is attached to a support material.

7. A method according to claim 6, in which attachment is by a biotin/streptavidin type interaction.

8. A method according to claim 1, in which the complex is formed by adding the probe and marker to the single strand in an appropriate buffer solution.

9. A method according to claim 8, in which the buffer solution is Hepes buffer.

10. A method according to claim 1, using a fluorescent intercalating dye, in which the dye is SYBR Green I.

11. A method according to claim 1, in which the single strand is derived from a double stranded DNA product of PCR amplification of a target sequence.

12. A method according to claim 11, in which the PCR product is at least 100 base pairs in length.

13. A method according to claim 11, in which the PCR product is from 40 to 100 base pairs in length.

14. A method of detecting DNA variation which comprises bringing together:

(a) a single strand of a DNA sequence containing the locus of a variation,

(b) an oligonucleotide or DNA analogue probe specific for one allele of the variation and capable of hybridizing to the single strand (a) to form a duplex,

(c) a marker specific for the duplex structure of (a) plus (b) which forms a complex with the said duplex and reacts uniquely when interacting within the duplex,

wherein:

(1) bringing together the components (a), (b) and (c) under conditions in which either

(i) the component (a) hybridizes to component (b) and the complex is formed with component (c), or

(ii) the components (a) and (b) do not hybridize and the complex with component (c) is not formed,

(2) thereafter steadily and progressively adjusting the conditions of the environment, respectively, either

(i) to denature the formed duplex and cause dissociation of the complex, or

(ii) to cause formation of the duplex and resulting complex,

(3) continually measuring an output signal indicative of the extent of hybridization of (a) and (b) and resulting complex formation with (c), and

(4) recording the conditions in which a change of output signal occurs which is indicative of, respectively,

(i) dissociation of the complex, or

(ii) formation of the complex.

15. A method according to claim 14 which comprises

(1) forming a series of two or more complexes of the kind defined, each with a probe specific for a different allele of the variation, and

(2) observing their respective denaturing or annealing conditions (e.g. denaturing or annealing temperatures) so as to distinguish alleles of the variation plus the homozygous or heterozygous state if appropriate.

16. A method according to claim 14, in which the marker is one which fluoresces when intercalated in double stranded DNA.

17. A method according to claim 16, in which the denaturing or annealing point is determined by reference to the first derivative of the fluorescence measurement curve.

18. A method according to claim 16, in which denaturing or annealing point is determined by reference to the second derivative of the fluorescence measurement curve.

19. A method according to claim 14, in which the single strand is attached to a support material.

20. A method according to claim 19, in which attachment is by a biotin/streptavidin type interaction.

21. A method according to claim 14, in which the complex is formed by adding the probe and marker to the single strand in an appropriate buffer solution.

22. A method according to claim 21, in which the buffer solution is Hepes buffer.

23. A method according to claim 14, using a fluorescent intercalating dye, in which the dye is SYBR Green I.

24. A method according to claim 14, in which the single strand is derived from a double stranded DNA product of PCR amplification of a target sequence.

25. A method according to claim 24, in which the PCR product is at least 100 base pairs in length.

26. A method according to claim 24, in which the PCR product is from 40 to 100 base pairs in length.

27. A method of detecting DNA variation which comprises:

(1) forming a complex consisting of:

(a) a single strand of a DNA sequence containing the locus of a variation,

(b) an oligonucleotide or DNA analogue probe specific for one allele of the variation hybridized to the single strand (a) to form a duplex, and

(c) a marker specific for the duplex structure of (a) plus (b) and which reacts uniquely when interacting within the duplex, and

(2) continually measuring an output signal of the extent of the resulting reaction of the marker and the duplex while steadily increasing the denaturing environment containing the complex,

(3) recording the conditions at which a change in reaction output signal occurs (herein termed the denaturing point) which is attributable to dissociation of the complex and is thereby correlated with the strength with which the probe (b) has hybridized to the single strand (a).

28. A method according to claim 27, which comprises

(1) forming a series of two or more complexes of the kind defined, each with a probe specific for a different allele of the variation, and

(2) observing their respective denaturing or annealing conditions (e.g. denaturing or annealing temperatures) so as to distinguish alleles of the variation plus the homozygous or heterozygous state if appropriate.

29. A method according to claim 27, in which the marker is one which fluoresces when intercalated in double stranded DNA.

30. A method according to claim 29, in which the denaturing or annealing point is determined by reference to the first derivative of the fluorescence measurement curve.

31. A method according to claim 29, in which denaturing or annealing point is determined by reference to the second derivative of the fluorescence measurement curve.

32. A method according to claim 27, in which the single strand is attached to a support material.

33. A method according to claim 32, in which attachment is by a biotin/streptavidin type interaction.

34. A method according to claim 27, in which the complex is formed by adding the probe and marker to the single strand in an appropriate buffer solution.

35. A method according to claim 34, in which the buffer solution is Hepes buffer.

36. A method according to claim 27, using a fluorescent intercalating dye, in which the dye is SYBR Green I.

37. A method according to claim 27, in which the single strand is derived from a double stranded DNA product of PCR amplification of a target sequence.

38. A method according to claim 37, in which the PCR product is at least 100 base pairs in length.

39. A method according to claim 37, in which the PCR product is from 40 to 100 base pairs in length.

40. A method of detecting DNA variation which comprises:

(1) bringing together:

(a) a single strand of a DNA sequence containing the locus of a variation,

(b) an oligonucleotide or DNA analogue probe specific for one allele of the variation and capable of hybridizing to the single strand (a) to form a duplex,

(c) a marker specific for the duplex structure of (a) plus (b) and which reacts uniquely when interacting within the duplex,

the components (a), (b) and (c) being brought together prior to formation of the defined complex and under conditions in which (a) and (b) do not hybridize;

(2) steadily adjusting the conditions of their environment to cause formation of the duplex and resulting complex, and

(3) measuring an output signal indicative of the occurrence of hybridization of (a) and (b) (herein termed the annealing point).

41. A method according to claim 40, which comprises

(1) forming a series of two or more complexes of the kind defined, each with a probe specific for a different allele of the variation, and

(2) observing their respective denaturing or annealing conditions (e.g. denaturing or annealing temperatures) so as to distinguish alleles of the variation plus the homozygous or heterozygous state if appropriate.

42. A method according to claim 40, in which the marker is one which fluoresces when intercalated in double stranded DNA.

43. A method according to claim 42, in which the denaturing or annealing point is determined by reference to the first derivative of the fluorescence measurement curve.

44. A method according to claim 42, in which denaturing or annealing point is determined by reference to the second derivative of the fluorescence measurement curve.

45. A method according to claim 40, in which the single strand is attached to a support material.

46. A method according to claim 45, in which attachment is by a biotin/streptavidin type interaction.

47. A method according to claim 40, in which the complex is formed by adding the probe and marker to the single strand in an appropriate buffer solution.

48. A method according to claim 47, in which the buffer solution is Hepes buffer.

49. A method according to claim 40, using a fluorescent intercalating dye, in which the dye is SYBR Green I.

50. A method according to claim 40, in which the single strand is derived from a double stranded DNA product of PCR amplification of a target sequence.

51. A method according to claim 50, in which the PCR product is at least 100 base pairs in length.

52. A method according to claim 50, in which the PCR product is from 40 to 100 base pairs in length.

53. A system for analyzing the results of one or more tests carried out on a plurality of samples to determine whether or not the samples meet at least one criterion, including

(1) a memory for storing data from an experiment on the samples in a database,

(2) a settable graphical test probe, and

(3) processing means for applying the test probe to the stored data.

54. A system according to claim 53, including a display for displaying in graphical form an indication of the samples tested and for indicating in said graphical display those samples falling within the defined test probe and those falling outside the defined test probe.

55. A system according to claim 54, including a selector for selecting on the display samples to be tested by a probe.

56. A system according to claim 53, including threshold setting means for setting a threshold value below which experimental data is excluded from further analysis.

57. A system according to claim 53, including a display operable to display in real time the results of an experiment carried out on a sample.

58. A system according to claim 53, wherein the processing means is operable to quality score each test sample with relation to a sample determined to produce the best test result.

59. A system according to claim 53, wherein the processing means is designed to analyze the results of a DASH or McSNP experiment.

60. A method of analyzing the results of one or more tests carried out on a plurality of samples to determine whether or not the samples meet at least one criterion, including the steps of

(1) storing data from an experiment on the samples in a database,

(2) setting a graphical test probe, and

(3) applying the test probe to the stored data.

61. A method according to claim 60, including the step of displaying in graphical form an indication of the samples tested and indicating in said graphical display those samples falling within the defined test probe and those falling outside the defined test probe.

62. A method according to claim 60, including the step of selecting on a display samples to be tested by a probe.

63. A method according to claim 60, including the step of setting a threshold value below which experimental data is excluded from further analysis.

64. A method according to claim 60, including the step of displaying in real time the results of an experiment carried out on a sample.

65. A method according to claim 60, including the step of quality scoring each test sample with relation to a sample determined to produce the best test result.

66. A method according to claim 60 for analyzing the results of a DASH or McSNP experiment.